Effect of Seasonality and Perisulfakinin on Engorgement by *Tabanus nigrovittatus* (Diptera: Tabanidae) in the Laboratory

K. E. DOWNER,[1,2] R. J. NACHMAN,[3] AND J. G. STOFFOLANO, JR.[1]

Ann. Entomol. Soc. Am. 100(2): 251–256 (2007)

ABSTRACT The horse fly *Tabanus nigrovittatus* Macquart (Diptera: Tabanidae), a hematophagous insect, is a nuisance pest along the Atlantic Coast. A description of the engorgement pattern throughout the season is lacking in the literature for this species. The percentage of flies engorging a bloodmeal in the laboratory throughout the season was recorded, and here we demonstrate that the percentage of flies that are blood feeding fluctuates, leading to a decrease in flies engorging as the season ends. Additionally, three recent nonhematophagous insect studies demonstrated that sulfakinins, a vertebrate homologue of cholecystokinin, function in feeding inhibition as a satiety factor. We found that groups of flies injected with one nanomole of perisulfakinin were inhibited from blood feeding by 45–60%. The satiation of feeding reported here is in agreement with the previous research by using nonhematophagous species. When groups of flies were injected with 10 nmol of perisulfakinin, the percentage of flies engorging was increased relative to the sham-injected flies, although not significantly. The stimulation of engorgement by sulfakinin has not previously been demonstrated, and its mode of action remains unclear.

KEY WORDS satiety, engorgement, sulfakinins, neuropeptides, cholecystokinin

Tabanus nigrovittatus Macquart (Diptera: Tabanidae) is an ideal hematophagous model to study feeding behavior because these flies can be collected in extremely large numbers on marshes, and considerable information already exists on phagostimulants (Friend and Stoffolano 1983, 1984; Friend 1991), food diversion, and feeding methods (Stoffolano 1979, 1983). Little is known about the natural engorgement pattern throughout the 3- to 4-wk season of these flies, especially regarding aging.

To date, the least understood aspects of *T. nigrovittatus* biology are the factors affecting satiety. Most of the female hematophagous insects studied seem to rely on the abdominal stretch receptors as the primary feedback mechanism for terminating blood-feeding and host-seeking behaviors (Gwadz 1969, Hocking 1971, Rice 1972, Friend and Smith 1977, Adams 1999). Specifically, the effects of chemical satiety factors have not been investigated.

Sulfakinins (SKs) are a family of invertebrate neuropeptides that are physiologically and structurally homologous to vertebrate cholecystokinin (Schoofs and Nachman 2006); thus, they play a role in satiation. Recent studies reported that sulfakinins reduce meal size and thereby significantly inhibit food intake by 50% (at 1 nmol, Wei et al. 2000) in the locust *Schistocerca gregaria* Förskal; by 60% (at 10 μg, Maestro et al. 2001) in the German cockroach, *Blattella germanica* (L.); and by 44% (at 10 nmol, Downer et al. 2007) in the female black blow fly, *Phormia regina* (Meigen). In *T. nigrovittatus*, sulfakinin-immunoreactive cells have been found in the brain, abdominal ganglion, and endocrine cells of the gut where the foregut and midgut merge (Haselton 2005). The goal of the current study is to investigate what role sulfakinins have on blood feeding and to determine what the natural pattern of engorgement of a population of *T. nigrovittatus* is over the season.

Materials and Methods

Collecting and Maintaining Flies. Female host-seeking *T. nigrovittatus* were field collected from box traps and brought back to the laboratory, as described by Downer and Stoffolano (2006), during July 2004 and 2005. Before experimentation, all flies were deprived of granulated sucrose for 16–20 h. All flies used in experimentation were tested the day after being collected in the field. The exact chronological ages of all flies used are unknown; however, during the 2005 field season the first observation of *T. nigrovittatus* in surveillance traps (three flies) occurred on 22 June 2005. The first collection date for the 2005 experiments occurred on 4 July 2005, and field collections were made every other day consecutively throughout the season until the flies were no longer on the marsh. Flies were only collected once a week during 2004.

Seasonality and Engorgement. Stoffolano (1979) demonstrated that horse flies could be blood fed in the

[1] Department of Plant, Soil and Insect Sciences, Division of Entomology, University of Massachusetts, Amherst, MA 01003.
[2] Corresponding author, e-mail: kelley.e.downer@aphis.usda.gov.
[3] Areawide Pest Management Research, Southern Plains Agricultural Research Center, USDA–ARS, College Station, TX 77845.

laboratory by using two methods. The parafilm membrane-feeding technique uses the probing mechanism of the fly, whereas the Kimwipe feeding mechanism does not require probing through a membrane and has been shown to be a more successful method for blood feeding in the laboratory. Thus, the percentage of flies engorging throughout the 2005 season was recorded for both feeding techniques.

All flies were cold immobilized in the freezer, and only flies of approximately the same size were used. A group (n = 40 in 2004; n = 20 in 2005) of flies was placed in a 23-cm^3 metal-screened cage, and the feeding assay was started. Each cage is a unit of replication. The feeding assay is described below.

In total, 1,315 flies were used for the seasonal engorgement studies with 34 replicates performed for the parafilm membrane feeding technique throughout both field seasons (2004 and 2005) and 23 replicates performed for the Kimwipe feeding technique in 2005. All dates in 2005 had a minimum of two replicates per date and a maximum of four replicates per date for each feeding technique. All statistical comparisons were performed using analysis of variance (ANOVA) to compare the percentage of engorged flies by date, and a Tukey–Kramer honestly significant difference (HSD) test was used in post hoc comparison (JMP, SAS Institute 2005).

Injection Technique for Perisulfakinin (PSK) Experiments. All flies were cold immobilized in the freezer, and only flies of approximately the same size were used. Flies were then placed in a petri dish on ice to prevent them from moving before injections. Sham-injected and treatment flies were injected with 1 μl in the second to last intersegmental membrane on the right ventral side of the abdomen. All flies were injected using a 30-gauge needle attached to a 25-μl glass gastight Hamilton #1750 syringe (Hamilton Co., Reno, NV). Sham-injected flies were injected with *Phormia* saline (Chen and Friedman 1975), and treatment flies were injected with 1 and 10 nmol of PSK [H-Glu-Gin-Phe-Asp-Asp-Tyr(SO_3H)-Gly-His-Met-Arg-Phe-NH_2] (Bachem, King of Prussia, PA) dissolved in *Phormia* saline. The nanomolar doses were chosen based on the previous insect studies with sulfakinins (Wei et al. 2000, Maestro et al. 2001, Downer et al. 2007). The sulfakinin was prepared in a stock solution of 80% acetonitrile and 20% water, made up to 0.01% trifluoroacetic acid. PSK, a cockroach sulfakinin, was used in these studies because a dipteran sulfakinin had not been synthesized in time for these particular experiments. The cockroach and fly sulfakinins share a common C-terminal heptapeptide sequence; the differences in structure between the cockroach and fly peptides are in the N terminus. Depending on the receptor, the differences in the N terminus may have an important effect on the structure–activity relationship, but they have not been tested. Using a dipteran sulfakinin may result in a greater observed effect on the feeding behavior of the horse fly.

After an individual fly was injected, it was placed back on ice until the entire experimental group (n = 40 for 2004; n = 20 for 2005) was completed. The entire injection process for each experimental group (i.e., sham or treatment) took <10 min. Each group of flies was placed in a 23-cm^3 metal-screened cage, and the feeding assay was started. There was zero mortality with all injections. All flies recovered from injections, and resumed normal "fly behavior" of walking, grooming, and so on. The recovery period took <5 min.

Feeding Assay. Friend and Stoffolano (1983) found that tabanids only successfully blood fed in a group setting of more than five flies, so all flies were group fed. Citrated beef blood was warmed on a hot-plate to 37°C and stirred with a magnetic stirrer. For the parafilm membrane feeding technique, plastic deli cups were fitted with a parafilm membrane and prepared as described by Downer and Stoffolano (2006). Warmed blood was poured into each cup, and a lamp with a 60-W bulb was positioned over it to provide adequate light and keep the blood warmed. For the blood-soaked Kimwipe feeding technique (used in the seasonality experiments), Kimwipes were placed on top of the cages, and warmed blood was pipetted onto the Kimwipes until they were thoroughly soaked. Only the parafilm membrane feeding technique was used in the PSK experiments. The flies were then allowed to feed ad libitum for 1 h. After the feeding assays were completed, flies were killed, and their midguts checked for the presence of a bloodmeal.

In total, 1,200 flies were used in the sulfakinin study, and 15 replicates were performed throughout the study (five replicates in 2004 and 10 replicates in 2005). One replicate consisted of a single run of all of the experimental cages (sham and sulfakinin-injected) simultaneously. All statistical comparisons were performed using ANOVA to compare the arcsine transformations of the percentage of engorged flies by treatment, and a Tukey–Kramer HSD test was used in post hoc comparison (JMP, SAS Institute 2005). The percentage of difference between the sham-injected group and the treatment group was calculated by: [(% engorged by treatment − % engorged by sham)/% engorged by sham] × 100.

Results

Seasonality and Engorgement. During the 2004 and 2005 field season, we recorded the percentage of females engorging through parafilm membranes throughout the season. There were two peaks (13 and 19 July) in the engorgement behavior during the middle of their season in 2005 (Fig. 1). Toward the end of both seasons, a lower percentage of flies engorged compared with "peak" season flies. There was a statistical difference in the engorgement response using parafilm membranes throughout the 2005 season ($F_{10, 17}$ = 2.58; P = 0.04). Data for 2004 was not statistically analyzed because there were not enough replicates for each date. The percentage of flies engorging was similar at approximately the same times during the month in both years. A lower percentage of flies engorged at the end of the season with a higher percentage of flies engorging during the peak

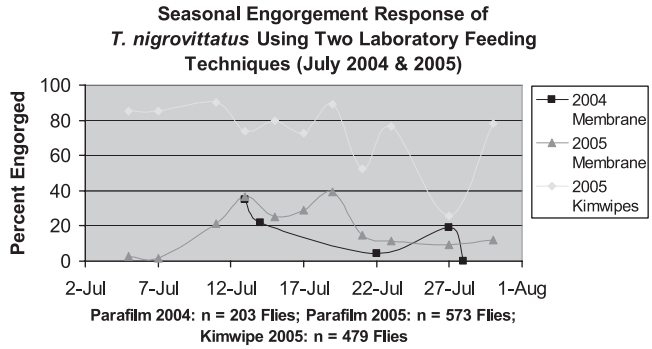

Fig. 1. Seasonal engorgement response by *T. nigrovittatus* when blood fed in the laboratory by using parafilm membrane (July 2004 and 2005) and blood-soaked Kimwipes (July 2005). Data points are similar for flies fed using the parafilm membrane technique for 2004 and 2005, suggesting that the females have a similar pattern of engorgement. In both the beginning and end of the season, the percentage of flies engorging is lower compared with the middle of the season. The engorgement patterns between the two different laboratory methods of blood feeding (for 2005) express similar peaks and valleys throughout the season; however, the flies exposed to blood using the Kimwipe feeding technique engorge at higher percentages. In addition, the flies exposed to blood by using the Kimwipe feeding technique do not express lower percentages of engorgement in the beginning of the season compared with flies exposed to blood by using the parafilm membrane feeding technique.

season, when fed through parafilm membranes (especially for 2005).

For the Kimwipe feeding technique, there was a significant difference in the percentage of females engorging throughout the season ($F_{10, 12} = 5.07$; $P = 0.005$) (Fig. 1). The peaks and valleys over the season for flies exposed to blood-soaked Kimwipes are not as pronounced throughout the season as they were for flies exposed to parafilm membranes.

Perisulfakinin and Satiety. There was a statistical difference with the effect of PSK on blood feeding during the 2004 field season ($F_{2, 10} = 4.24$; $P = 0.046$; Fig. 2). However, only the 10- and 1-nmol doses were significantly different from one another. Neither treatment mean was significantly different from the sham-injected mean. Of the grouped flies that were sham injected, 37% engorged a bloodmeal. There was a nearly doubled increase in the percentage (63%) of flies that engorged a bloodmeal when injected with a high dose of PSK (10 nmol). There was a lower percentage (15%) of flies that engorged when injected with a low dose (1 nmol) of PSK.

During the 2005 field season, there was no statistical difference detected for the effect of PSK on engorgement ($F_{2, 27} = 0.722$; $P = 0.495$) (Fig. 3). The 2005 data were obtained under more controlled conditions (e.g., flies were collected every other day from the traps). Of the grouped flies that were sham injected, 11% engorged a bloodmeal. There was a higher percentage (16%) of flies that engorged a bloodmeal when injected with a high dose of PSK (10 nmol), whereas there was a lower percentage (6%) of engorged flies when injected with 1-nmol doses of PSK. Importantly, the pattern of engorgement remained the same for both the 2004 and 2005 field seasons, with 10-nmol injections increasing the percentage of flies engorging and 1-nmol injections decreasing the percentage of flies engorging a meal.

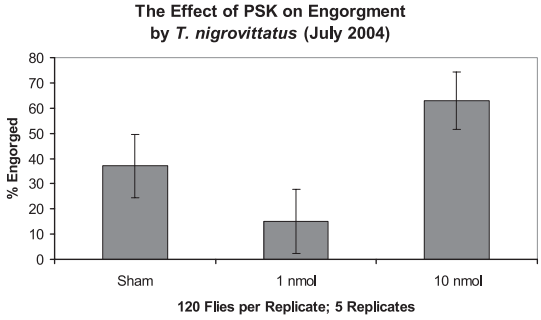

Fig. 2. Effect of PSK on engorgement by *T. nigrovittatus* in 2004 ($F_{2, 10} = 4.24$; $P = 0.046$). The percentage of flies engorged was 63 ± 11.33 (mean ± SEM) when injected with 10 nmol of PSK. Only 15 ± 12.67% of flies engorged when injected with 1 nmol. The percentage of flies engorged for the sham treatment was 37 ± 12.67). In total, 600 flies were used with 120 flies per replicate and five replicates performed. Small bars represent SEM.

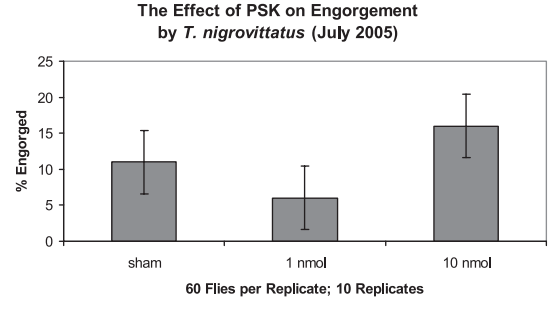

Fig. 3. Effect of PSK on engorgement by *T. nigrovittatus* in 2005 ($F_{2, 27} = 0.722$; $P = 0.495$). The percentage of flies engorged was 16 ± 4.39 when injected with 10 nmol, but only 6 ± 4.39% of flies engorged when injected with 1 nmol. The percentage of flies engorged for the sham treatment was 11 ± 4.39%. In total, 600 flies were used with 60 flies per replicate and 10 replicates performed. Small bars represent SEM.

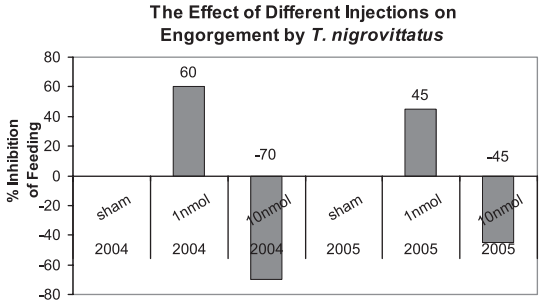

Fig. 4. Effect, expressed here as inhibition or stimulation, of PSK on engorgement by *T. nigrovittatus*. The peptide was injected at 1 nmol per fly and 10 nmol per fly. In 2004, 1-nmol injections of PSK inhibited engorgement by 60%, whereas 10-nmol injections stimulated engorgement by 70%, compared with the sham-injected group. In 2005, injections of 1 nmol of PSK reduced the percentage of flies gorging by 45%, whereas injections of 10 nmol of PSK increased the percentage of flies gorging through an artificial membrane by 45%, compared with the sham-injected flies.

Previous research (Wei et al. 2000, Maestro et al. 2001) reported data as the percentage of feeding inhibition. If we examine the data as the percentage of inhibition of engorgement, 1 nmol of PSK inhibited feeding by 60% and 10 nmol of PSK stimulated feeding by 70% (2004) (Fig. 4). For the 2005 experiments, 1 nmol of PSK inhibited feeding by 45% and 10 nmol of PSK stimulated feeding by 45% (Fig. 4).

Discussion

Seasonality and Engorgement. A review of the literature revealed that there is no description of seasonal probing or engorgement (and ultimately aging) patterns for *T. nigrovittatus*. Knowing the normal percentage of engorgement throughout the season would be useful to predict an inhibiting effect from sulfakinins on engorgement as well as for other hematophagous studies. Additionally, notes on emergence for this species would be useful for knowing whether the same flies that emerged in the beginning of the season are on the marsh all 3–4 wk of the season, whether they have a peak in probing activity, and whether aging contributes to a decrease in probing because of physiological or endocrinological degradation, or both.

The results of this study demonstrate an increase in engorgement in *T. nigrovattus* as the season peaks and a decrease in engorgement as the season ends. There was a high percentage of feeding early in the season with a gradual and inconsistent decline over the season for flies exposed to blood by using Kimwipes. This finding contrasts with the steady increase in engorgement up to and during peak season and the decline toward the end of the season for flies fed using parafilm membrane. It is possible that it is easier for young, naïve flies to obtain blood from Kimwipes, rather than learning to penetrate the parafilm. Both methods are artificial laboratory techniques and may not represent actual engorgement behavior. Presumably, all flies that enter the box traps in the field are in the blood-feeding mode. Why then are the engorgement rates different throughout the season? Senescence in *T. nigrovittatus* may influence host-seeking behavior differently from engorgement behavior. We suspect that as the season progresses and the fly populations begin to age on the marsh, older flies experience a physiological degradation of chemoreceptors (Stoffolano et al. 1978), become less responsive to stimuli, and less able to execute probing and ingestion. In addition, because hormones have been shown to effect probing in other hematophagous Diptera (*Culex* spp., Meola and Petralia 1980), aging *T. nigrovittatus* probably also experience a change in hormone levels, which could account for the lack of engorgement later in the season. The effect of aging on engorgement also has been demonstrated by Mather and DeFoliart (1984) who showed that older female mosquitoes had reduced feeding success on live hosts.

The low engorgement at the end of the season makes biological sense in relation to what the normal aging patterns probably are in the horse fly. The first collection of *T. nigrovittatus* in the marsh traps was on 4 July 2005. Most likely, these flies emerged sometime in the end of June, mated, and females laid eggs [oogenesis taking 7–10 d, Magnarelli and Stoffolano (1980)] and were seeking out their first bloodmeal, consequently putting them in the box traps. Based on observations made through dissections, none of the flies used in the beginning of the season had previously engorged before experimentation. On 27 July 2005, we noted the first instances of flies that had produced at least two batches of eggs before being caught in the traps. This deduction was based on conditions of the ovarioles, the distended midguts, and in several instances, the leftover remnants of bloodmeals in the midgut. Counting back 10 d earlier (for oogenesis) means the flies would have mated and engorged a bloodmeal ≈17 July 2005. The females probably first mated and produced a batch of eggs another 10 d before that (7 July 2005) without a bloodmeal because they are autogenous, putting their emergence somewhere in the first week of July.

Furthermore, survivorship curves performed in the laboratory by Stoffolano and Majer (1997) showed that *T. nigrovittatus* could live for ≈20 d after engorgement, but the mean survivorship was ≈9 d for blood-fed females. Thompson and Krauter (1978) showed similar results with <10% of the population surviving past 15 d in the laboratory, and 0% survivorship after ≈25 d in the laboratory. Therefore, it is entirely possible that the flies that emerged at the end of June and the first week of July are the same flies on the marsh at the end of July (i.e., toward the end of their season).

Perisulfakinin and Satiety. This study examined the effect of sulfakinin in a natural hematophagous insect population. Using a field-collected population has limitations, particularly in that the physiological and chronological age remains unknown, both of which can cause variability in the data and are most likely responsible for the large amount of variation within

our data. Perisulfakinin has a dose-dependent effect on the gorging behavior of *T. nigrovittatus*, with 10 nmol stimulating feeding and 1 nmol inhibiting feeding, although not significantly against the sham-injected flies.

We have demonstrated an inhibition of engorgement in this species (60% in 2004 and 45% in 2005) at the 1-nmol dose of PSK. Our finding that sulfakinin has a satiating effect on feeding is in agreement with the results reported by Wei et al. (2000), Maestro et al. (2001), and Downer et al. (2007). Maestro et al. (2001) used sulfakinin doses of 0.1, 1, and 10 μg per cockroach, with 10 μg being the most effective dose. Wei et al. (2000) used doses of 10 pmol–1 nmol per locust and found that 1 nmol was the most effective dose. Downer et al. (2007) used doses of 1–10 nmol per blowfly and found that 10 nmol was the most effective dose in reducing feeding. This study has shown that the 1-nmol dose was the most effective in the inhibition of engorgement. The stimulation of engorgement is unexpected and has not been demonstrated previously in another insect with sulfakinin. It is possible that 10-nmol injections of PSK are increasing engorgement behaviorally by affecting probing through either a neurological pathway or physiologically through a water deficit pathway.

Wei et al. (2000) and Maestro et al. (2001) reported insects taking meals but in smaller meal sizes. The cockroach and locust are continual feeders. When left undisturbed, *T. nigrovittatus* feeds to repletion and only takes one bloodmeal between each gonadotrophic cycle. We did not measure the amount of blood ingested for this reason, and instead we measured the percentage of females engorged. Thus, it is interesting that low doses of sulfakinin deterred flies from taking an entire meal, opposed to regulating the size of the meal, as was shown by Wei et al. (2000), Maestro et al. (2001), and Downer et al. (2007). The deterrence of flies from taking a meal raises the question of whether sulfakinin is specifically affecting the probing mechanism because the size of the bloodmeal has been shown to be regulated by stretch receptors in other hematophagous insects (Gwadz 1969).

The differences in the percentages of engorgement between the two field seasons in our study are most likely due to how long the flies were in the traps on the marsh. In 2004, flies were collected once a week, whereas in 2005 they were collected every other day. It has been shown in another hematophagous fly, the tsetse fly, that starvation changes the feeding thresholds so that a more starved fly will elicit a greater probing response (Brady 1973). The difference between the shams (37% in 2004 versus 11% in 2005) suggests that starvation, thirst, or both could account for the higher engorgement in 2004 because flies were in the traps longer. Thus, the 2005 data may be more indicative of the flies' response to PSK given their physiological condition was more controlled. Using a dipteran sulfakinin instead of a cockroach sulfakinin may elicit a more significant statistical effect; however, it is evident that 1 nmol of sulfakinin has a biological effect on blood feeding as an inhibition factor by reducing the percentage of flies engorging by 45–60%.

Acknowledgments

We are very thankful to Jack Card, Esteban Cuebas-Incle, and Walter Montgomery of the Northeast Massachusetts Mosquito Control and Wetlands Management District for expertise, knowledge, and use of field traps. We also thank Rick Adams of Adam's Farm (Athol, MA) for supplying fresh bovine blood, Lynn Adler and Eva Goldwater for statistical consulting, and Jerrold Meyer, Eric Corp, and George Wade for helpful insight and discussion. We acknowledge the constructive criticism on this manuscript from two anonymous reviewers. This research was supported by the Massachusetts Agricultural Experiment Station (Hatch 817 to J.G.S.). This article is contribution 3383 from the Massachusetts Agricultural Experiment Station.

References Cited

Adams, T. S. 1999. Hematophagy and hormone release. Ann. Entomol. Soc. Am. 92: 1–13.

Brady, J. 1973. Changes in the probing responsiveness of starving tsetse flies (*Glossina morsitans* Westw.) (Diptera, Glossinidae). Bull. Entomol. Res. 63: 247–255.

Chen, A., and S. Friedman. 1975. An Isotonic saline for the adult blowfly, *Phormia regina*, and its application to perfusion experiments. J. Insect Physiol. 21: 529–536.

Downer, K. E., and J. G. Stoffolano, Jr. 2006. The effect of octenol on engorgement by the salt marsh horse fly, *Tabanus nigrovittatus* Macquart (Diptera: Tabanidae). J. Med. Entomol. 43: 643–645.

Downer, K. E., A. T. Haselton, R. J. Nachman, and J. G. Stoffolano, Jr. 2007. Insect satiety: sulfakinin localization and the effect of drosulfakinin on protein and carbohydrate feeding in the blow fly, *Phormia regina* (Diptera: Calliphoridae). J. Insect. Physiol. 53: 106–112.

Friend, W. G., and J.J.B. Smith. 1977. Factors affecting feeding by bloodsucking insects. Annu. Rev. Entomol. 22: 309–331.

Friend, W. G., and J. G. Stoffolano, Jr. 1983. Feeding responses of the horse fly, *Tabanus nigrovittatus*, to phagostimulants. Physiol. Entomol. 8: 377–383.

Friend, W. G., and J. G. Stoffolano, Jr. 1984. Feeding responses of the horse fly, *Tabanus nigrovittatus*, to physical factors, ATP analogues and blood fractions. Physiol. Entomol. 9: 395–402.

Friend, W. G. 1991. Feeding behavior of the horse fly, *Tabanus nigrovittatus* (Diptera: Tabanidae): effects of dissolved solids on ingestion and destination of sucrose or ATP diets. Physiol. Entomol. 16: 35–45.

Gwadz, R. 1969. Regulation of blood meal size in the mosquito. J. Insect Physiol. 15: 2039–2044.

Haselton, A. T. 2005. Myotropic peptide hormones and serotonin in the regulation of feeding in the adult blow fly *Phormia regina*, and the adult horse fly *Tabanus nigrovittatus.* Ph.D. dissertation, University of Massachusetts, Amherst, MA.

Hocking, B. 1971. Blood-sucking behavior of terrestrial arthropods. Annu. Rev. Entomol. 16: 1–26.

Maestro, J., R. Aguilar, N. Pascual, M. L. Valero, M. D. Piulachs, D. Andreu, I. Navarro, and X. Bellés. 2001. Screening of antifeedant activity in brain extracts led to the identification of sulfakinin as a satiety promoter in the German cockroach. Eur. J. Biochem. 268: 5824–5830.

Magnarelli, L. A., and J. G. Stoffolano, Jr. 1980. Blood feeding, oogenesis, and oviposition by *Tabanus nigrovittatus* in the laboratory. Ann. Entomol. Soc. Am. 73: 14–17.

Mather, T. N., and G. R. DeFoliart. 1984. Reduced feeding success on squirrels and chipmunks by older *Aedes triseriatus* females. Mosq. News 44: 471–475.

Meola, R. W., and R. S. Petralia. 1980. Juvenile hormone induction of biting behavior in *Culex* mosquitoes. Science (Wash., D.C.) 209: 1548–1550.

Rice, M. J. 1972. Recent advances in physiology of tsetse flies and other hematophagous Diptera, Chapter 5, Paper 4, pp. 197–201. *In* C. Anderson and W. L. Kilama [eds.], Parasitoses of man and animals in Africa. Proceedings of the East African Medical Research Council Scientific Conference East African Literature Bureau.

SAS Institute. 2005. JMP Start statistics, 3rd ed. Brooks/Cole-Thomson Learning, Belmont, CA.

Schoofs, L., and R. J. Nachman. 2006. Sulfakinins, pp. 183–189. *In* A. Kastrin [ed.], Handbook of biologically active peptides. Elsevier, San Diego, CA.

Stoffolano, J. G., Jr. 1979. Comparison of methods for feeding blood to *Tabanus nigrovittatus* in the laboratory. Ann. Entomol. Soc. Am. 72: 216–217.

Stoffolano, J. G., Jr. 1983. Destination of the meal and the effect of a previous sugar or blood meal on subsequent feeding behavior in female *Tabanus nigrovittatus* (Diptera: Tabanidae). Ann. Entomol. Soc. Am. 76: 452–454.

Stoffolano, J. G., Jr., and J. Majer. 1997. Comparison between tsetse fly synthetic diet and bovine blood on reproduction and survivorship in *Tabanus nigrovittatus* (Diptera: Tabanidae). J. Med. Entomol. 34: 738–740.

Stoffolano, J. G., Jr., R. A. Damon, and C. E. Desch. 1978. The effect of age, sex, and anatomolical position on peripheral responses of taste receptors in blow flies, genus *Phormia* and *Protophormia.* Exp. Gerontol. 13: 115–124.

Thompson, P. H., and P. C. Krauter. 1978. Rearing of Texas Tabanidae (Diptera). I. Collection, feeding, and maintenance of coastal marsh species. Proc. Entomol. Soc. Wash. 80: 616–625.

Wei, Z., G. Baggerman, R. J. Nachman, G. Goldsworthy, P. Verheart, A. De Loof, and L. Schoofs. 2000. Sulfakinins reduce food intake in the desert locust, *Schistocerca gregaria.* J. Insect Physiol. 46: 1259–1265.

Received 16 March 2006; accepted 15 November 2006.